

FIG. 1. QUANTITATIVE DETERMINATION OF PENICILLIN BY THE METAL
METHOD METHOD IN PRODUCTION CONTROL USSR A. L.
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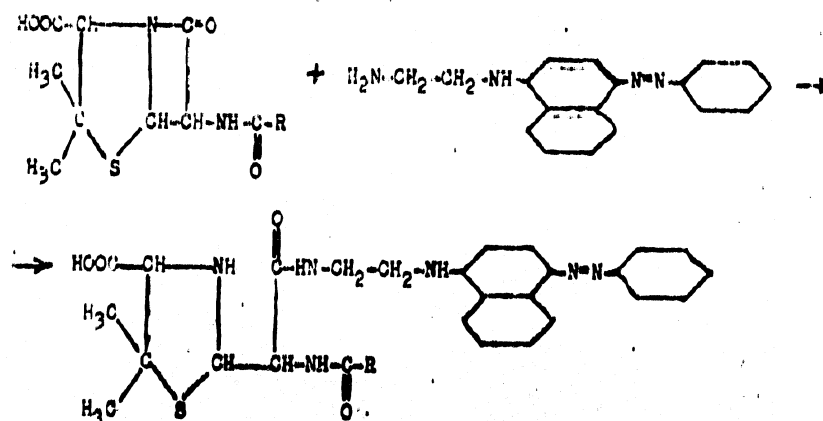
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The ~~amino acid~~ penicillin enter into a condensation reaction according to the following scheme:



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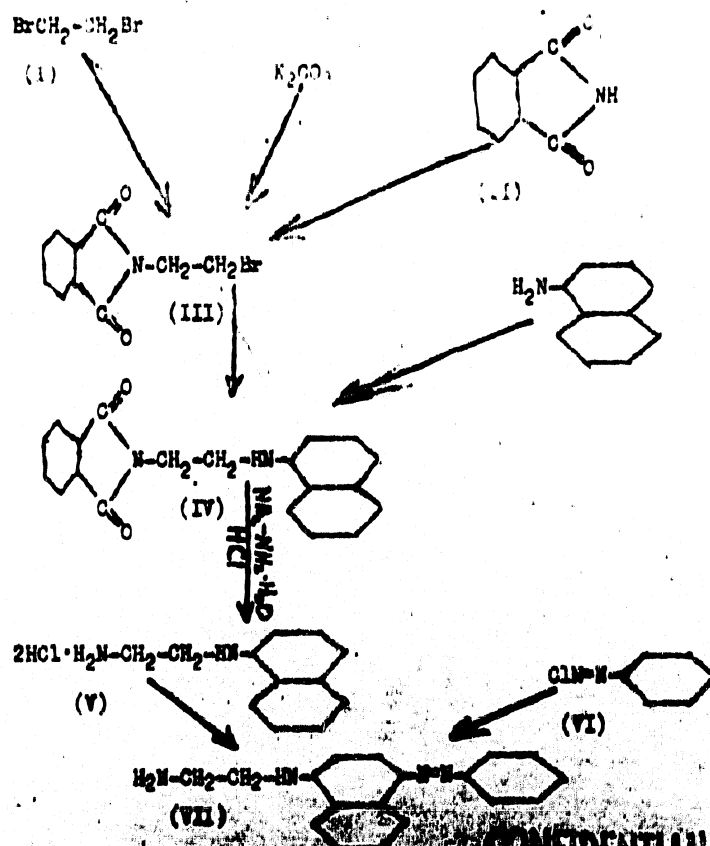
R designates the radical for the particular type of penicillin

The condensation product has an intense color, and penicillin ^{can be} quantitatively determined ^{by measuring the depth of this color} with the aid of a graduated photometer or an electrophotocolorimeter.

Although this method has many advantages over ~~the~~ other methods for the quantitative determination of penicillin, its general use ~~has~~ ^{has} been held up by the difficulties encountered in the synthesis of the reagent. Below are described: 1) a new, simple method for the synthesis of N-(1-naphthyl-4-azobenzene)-ethylene diamine; and 2) the procedure for ^{the} quantitative determination of penicillin by the colorimetric method, using this reagent.

1. Synthesis of N-(1-Naphthyl-4-Azobenzene)-Ethylene Diamine [by A. L. Yaroslavtsev]

The synthesis was carried out by the Gabriel method (3) as modified by Ing and Manske (4). The whole process can be represented by the following scheme:



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Dibromoethane (I) was synthesized in the usual manner by the Catterman method, and the fraction boiling at 129-130°C was used in the synthesis of β -bromoethylphthalimide.

Phthalimide (II) was prepared from phthalic anhydride and ammonium carbonate. For the synthesis of β -bromoethylphthalimide a pure preparation, recrystallized from water, was used.

β -Bromoethylphthalimide (III). 75 g of phthalimide and 37.5 g of nonaqueous potassium carbonate, well ~~and~~ pulverized and mixed in a mortar, were placed in a ^{500 ml} round-bottomed flask fitted with a mechanical agitator and two condensers, after which 250 g of dibromoethane ^{were} introduced. The flask was slowly heated ^{on} an oil bath, with the contents well agitated, until the temperature of the bath reached 130-140°, at which point the reaction mixture, originally free-flowing, solidified, ^{then} and the agitator was disconnected. The heating was continued at a bath temperature of 170-180° for 6-8 hours and the mixture finally became free-flowing, having the appearance of a brownish oil. The excess dibromoethane ^(130-140 g) was distilled off under a ~~vacuum~~ 10-20 mm vacuum or with steam. The viscous residue (solidifying on cooling) was washed 4-5 times with water to remove mineral salts. The residue in the flask was dissolved in 150 ml of alcohol by heating ^{on} a water bath for 30-40 min. and the hot solution was filtered with ^{suction} a Nutsche filter. ^{Light} Slightly brown-colored crystals formed on cooling. ^{These crystals consisted of} a mixture of β -bromoethylphthalimide and diphtalimidethane. The ^{crystals were} separated ^{by extracting} in a Soxhlet apparatus with petroleum ether (bp 40-60°) ^{for} 10-12 hours. The petroleum ether took up only β -bromoethylphthalimide which, after recrystallization from hot 75% alcohol and bleaching with a small amount of animal charcoal, was obtained in the form of colorless, needle-like crystals (mp 82-83.5°) with a yield of 60-70 g. No traces of β -bromoethylphthalimide were detected in the residue.

N-(1-Naphthylamino)-ethylphthalimide (IV) was synthesized basically by the Newman method (5), taking into account modifications by Bratton and Marshal (6). A mixture of 40 g of β -bromoethylphthalimide and 60 g of freshly recrystallized α -naphthylamine was carefully pulverized in a mortar, transferred to a flask, and heated on an oil bath to 160°. The dark brown viscous mass ^{then} formed was washed

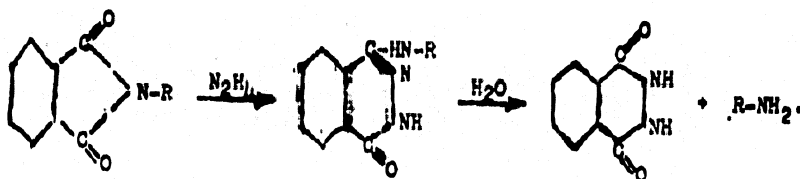
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with 1000 ml of hot water and then 50 ml of hot ~~95%~~ alcohol. The residue in the flask was washed with hot ~~95%~~ alcohol. When the alcoholic solution had cooled, an amorphous yellow powder was filtered out, dried, recrystallized from alcohol, and bleached with 3 g of animal charcoal. A yield of 25-30 g of golden-yellow crystals of β -(1-naphthylamino)-ethylphthalimide (mp 156°) was obtained.

Bratton and Marshal (6) recommend that the wet product be crystallized from glacial acetic acid. By this method golden-greenish crystals were obtained with mp 163-165° and a yield one and one half times as great as by recrystallization from alcohol.

N-(1-naphthyl)-ethylene diamine-dihydrochloride(V) can be prepared by splitting phthalic acid from ~~ethylphthalimide~~ β -(1-naphthylamino)-ethylphthalimide. This was accomplished by the Ing and Manske method, for which they propose the following scheme:



6.5 g of 50% hydrazine hydrate was added to a suspension of 20 g of β -(1-naphthylamino)-ethylphthalimide in 200 ml of alcohol. The mixture was boiled with a reflux condenser on a water bath for 3 hours. White flakes appeared at the end of the first hour, and after 1.5-2 hours the whole mass solidified. After the heating was completed, the white ~~residue~~ residue was hydrolyzed with 100 ml of ~~50%~~ HCl solution. Heating was continued for one more hour, after which the insoluble phthalylhydrazide was filtered off and washed with water, while the filtrate was concentrated by distilling off about 150 ml of alcohol. On cooling, phthalylhydrazide crystals were again formed and were filtered off. ~~(A strong solution)~~ ~~(A strong solution)~~ KOH was added to the ~~solid~~ solid filtrate until there was a clearly alkaline reaction. The amine was extracted with dry ether. The ether extract was dried over solid KOH and transferred to a flask, where dry HCl was passed over it ~~whereupon~~ ~~whereupon~~ N-(1-naphthyl)-ethylene diamine-dihydrochloride was formed as a white, flaky precipitate.

The precipitate was collected on a filter, quickly pressed between sheets of filter paper, and dried in a vacuum dryer. The yield of the wet product was 5-7 gr. After recrystallization from 6 N-HCl with activated carbon, white crystals of

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N-(1-naphthyl)-ethylenediamine-dihydrochloride ~~was~~ ^{mp} 162-163° (there was no stability ~~test~~ ^{test} were formed. When hydrazine-hydrate was replaced by an equivalent quantity of hydrazine sulfate with Na_2CO_3 and an insignificant quantity of water added, the hydrolysis ^{crude} proceeded smoothly, and satisfactory yields of the ~~crude~~ product were obtained.

N-(1-Naphthyl-4-azobenzene)-ethylenediamine (2) (VII). As a preliminary to the final synthesis of the reagent it was necessary to prepare the diazonium salt (VI). 5 g of freshly recrystallized aniline hydrochloride ~~was~~ ^{was} dissolved in 250 ml of water containing 6.4 ml of concentrated HCl. To this solution was added a solution of 3.2 g of NaNO_2 in 50 ml of water cooled to 5° by small drops. During the whole operation the temperature was kept at 0-5°. The diazotization was controlled with the aid of ~~iodine~~ ^{iodine}-starch paper. ~~(This test,~~ ^(with vigorous agitation) after the addition of a drop of the NaNO_2 solution, was taken every 15 minutes.) The resultant diazonium salt solution was poured in small quantities into a solution of 10 g of N-(1-naphthyl)-ethylenediamine-dihydrochloride in 500 ml of cold water containing 16 ml of concentrated HCl. The solution, colored dark violet, was left to stand 15 minutes at room temperature and then heated on a water bath to 60°, where it was held for 30 minutes. After it had cooled a concentrated NaOH solution was added by portions until the color changed from dark violet to orange. The addition of alkali was continued until the pH of the solution was approximately 9.6. The resultant precipitate was filtered out and dried. The ~~recrystallization~~ ^{yield} of wet product was about 6 g. After a two-stage recrystallization from 50% aqueous methanol the needle-like orange-colored crystals of N-(1-naphthyl-4-azobenzene)-ethylenediamine (mp 102-103°) were obtained.

2. Colorimetric Quantitative Determination of Penicillin [by Yaroslavtsev and Klimov]

The procedure for quantitative determination of penicillin by ~~condensation~~ ^{the} of the solution to be tested with N-(1-naphthyl-4-azobenzene)-ethylenediamine and the colorimetric determination of the resulting substance consists of the following. The penicillin solution to be determined is ~~added to~~ ^{added to} a glycine

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buffer solution and chloroform. The chloroform phase is separated, ~~and~~ dried with non-aqueous Na_2SO_4 , and a portion is mixed with a benzene solution of N-(1-naphthyl-4-azobenzene)-ethylenediamine and agitated with aqueous NaOH. The aqueous alkaline solution is extracted from the boundary layer between the upper benzene and lower ~~benzene~~ chloroform-benzene phases, and ~~concentrated~~ concentrated HCl added. The solution, ~~is~~ colored a crimson-red, ^{whose} intensity depending on the quantity of condensation product, is then treated with a mixture of butyl alcohol and benzene. After the phases have separated the aqueous layer is discarded and the butyl alcohol-benzene extract mixed with absolute alcoholic HCl. ~~The intensity of the color is then measured on a graduated photometer or a photoelectric colorimeter.~~

The reaction was first conducted with standard solutions of penicillin G containing quantities of penicillin varying from 10 to 120 μ . From the colorimetric values for each of these quantities a standard curve of light absorption against penicillin content was constructed.

By the ~~method~~ ^{determination of} method ~~described~~ ^{variations} above for the ~~method~~ standard penicillin solutions, although with minor ~~variations~~ in some cases, penicillin was determined successfully within the quantitative limits indicated above in urine, in the ~~separate stages of production of penicillin~~ end product of penicillin manufacture, and in the individual stages of penicillin production: i.e., the liquid in which the culture is produced, the ^{the} $\frac{m}{15}$ phosphate buffer, butylacetate extract, ~~the~~ the chloroform extract resulting from treatment of the buffer extract with chloroform, and ~~the~~ the bicarbonate solution ~~for production of the Na-salt.~~

Tabulated results of the determination of specimens of crystalline penicillin ~~noncrystalline~~ G, ~~the~~ a well purified, ~~noncrystalline~~ commercial preparation, and a commercial preparation ~~with~~ with a large content of "ballast" substances, showed that the results yielded by the colorimetric method ~~although they~~ were very sensitive, ~~in some cases~~ ^{although they} differed widely from results obtained by the iodometric and biological methods. However, discrepancies between results of separate determinations of the same penicillin preparation by the colorimetric method were very small, much smaller than by the biological method, ~~for example.~~

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